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Scraping Away at the Past: Extracting Ancient DNA from Stone Tools

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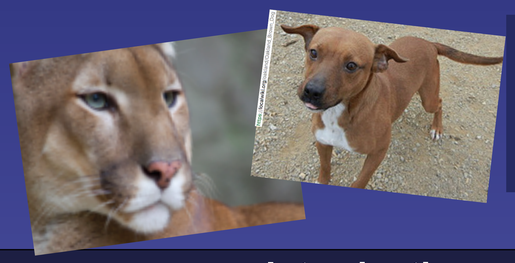

Scraping Away at the Past: Extracting Ancient DNA from Stone Tools

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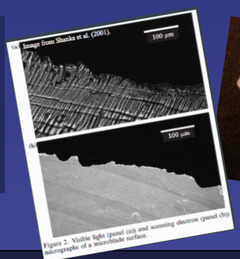


Figure 2. Visible light (panel (a)) and scanning electron (panel (b)) micrographs of a microblade surface.

Introduction:

The assemblages of lithic materials allow us to gain a better understanding as to what was going on at the archaeological sites where they are discovered. From chemical analyses to replicative tool manufacture studies, we are able to analyze the tools and learn more about the technological advances and subsistence strategies of those that used them. This research project is looking specifically at tools from the Bridge River site in the Middle Fraser Canyon of British Columbia, and their associated aDNA.

This site, occupied from 1,800 years B.P. until ~mid 19th century, has been preserved in the housepit sequential floors that have been excavated by Dr. Anna Prentiss of the Anthropology Department (Prentiss 2017; Prentiss et al. 2018).

Hypothesis:

A portion of our research is focused around perfecting the aDNA extraction methods for lithic artifacts' micro cracks. Additionally, we believe that our extraction and analysis of DNA from these tools will yield positive identification of the species on which these tools were used.

Goals:

- Improve aDNA extraction and amplification protocols for archaeologically associated lithics.
- Explore new possible results of this methodology and reassess how the findings contribute to our knowledge of the Bridge River site.

Background:

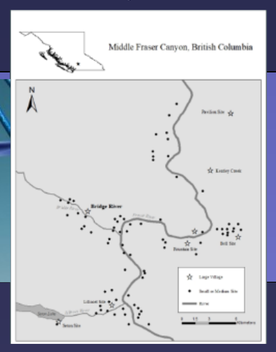
The Bridge River site, and House Pit 54 especially, were the focus of a large scale excavation that unearthed an extensive amount of lithic artifacts. These artifacts have since been analyzed for their unique characteristics, such as their use wear and source material. 326 tool types, 18 different use wear patterns, and 53 categories of raw material have been identified at the site.

Use wear directionality is a form of macrowear that then determines a tool's microenvironment. This effects where certain residues may become trapped, or if they adhere to the surface of the tool only. What they trap is determined by the amount of heat, friction, silica present, and of course the deposition of the artifact (Haslam 2006; Langejans 2010; Longo et al. 2005; Marreiros et al. 2015; Wadley et al. 2004; Wadley and Lombard 2007).

Microcracks, the byproducts of pressure and percussion from flaking techniques, can trap DNA within minutes of use (Shanks et al 2005). The extraction of the aDNA is relatively new in terms of residue analysis. However, their analysis is advantageous, as even if the surface of the tool is compromised, the DNA trapped inside is still obtainable and often well preserved. Due to the decay of DNA and contamination, successful extractions are rare and heavily debated. Nevertheless it is a viable option for learning more of a culture when no other organic material remains (Shanks et al. 2004).

Abstract:

This research project seeks to explain the use of lithics found at the Bridge River site in British Columbia through the extraction and analysis of ancient DNA (aDNA) found on the surface of stone tools. The methods used for extraction were nondestructive. Using sonication to release the trapped aDNA from microcracks on the tool's surface, and the amplification of mitochondrial DNA regions Cytochrome B and 16S in order to determine what species the tools were used to process. The findings of this project have the potential to further refine the extraction process for ancient DNA present on lithic material, as well as end archaeologists' longtime debate over whether or not certain tools were used explicitly for one particular organic material, such as with the making of bone tools, and whether or not scrapers were specifically used for one species at the Bridge River site. To date, we have worked with over 65 tools, and extracted both Puma (*Puma concolor*) and Dog (*Canis lupis familiaris*) DNA from our samples. This project provides us with a unique opportunity to both enhance our knowledge of lithic use at archaeological sites, and successfully extract more genetic material moving forward.



Challenges:

- **Degradation**
  - Ancient DNA is by nature low-quality due to the degradation of the DNA molecules that occurs. This degradation has been linked with time, temperature, water presence, etc. As the degradation worsens, the structure of the DNA deteriorates and we can be left with small chunks of sequence. (Pääbo et al. 2004; Burger 1999).
- **Contamination**
  - Despite all of the efforts made to mitigate it, it can still occur. Even if it's not from researchers, possibilities of false positives could be due to the DNA amplification reagents themselves, as they come from modern samples such as bacteria or livestock. Reagents yield false positives 2-5% of the time. Leonard et al. 2007)
  - Furthermore, a successful extraction may have aDNA that is difficult to prove as being from use. In order to prove findings correct, we must take the archaeological site's context into account Langejans 2010).
    - Using the same methodology as Shanks et al, other studies have had successful extractions only 10% of the time (Kimura et al. 2001), and we have had an even lower success rate of ~5.5%, perhaps because of the age of the DNA and site.
- **Tool Type**
  - Scrapers, which were our main lithic items, may not preserve aDNA well. This is due to sharpening, which leads to the loss of microcracks. However, we have still been able to get dog and puma DNA from them in our research (Kimura et al. 2001).

Acknowledgments:

I would like to thank Dr. Meradeth Snow, Dr. Anna Prentiss, Rachel Summers-Wilson, Clare Super, Ethan Ryan, Kristy Pilgrim, Nathan Goodale for their help in the lab and contributions to this project. We also thank our partner, Xwisten, the Bridge River Band for their significant role in facilitating this research.

Conclusions:

To date, we have been able to obtain the aDNA of *Puma concolor* and *Canis lupus familiaris* from 4 stone tools from the Bridge River site, all of which were slate scrapers. Our preliminary research suggests that it is possible to obtain DNA from lithics. The rate of success seen with these stone scrapers in particular indicates that they are successful at preserving DNA, and were most likely heavily used in the processing of these animal hides, or at least came into contact with these species during their usage.

Degradation, contamination and other complications are all aspects of our research that we have addressed and will continue to combat. While these things will always be a concern, the presence of both dogs and puma have been heavily documented at this site and it is believed that our findings are an accurate portrayal of lithic use at the Bridge river site.

Moving forward, we would like to shotgun sequence extracts from these tools, and obtain a better understanding as to whether or not there was mixture in the species they were used on.

Methods:

The methods used for aDNA extraction and analysis is based on Shanks et al (2001). All of the following was performed in the Snow Molecular Anthropology Ancient DNA lab in the Anthropology Department. Access to this lab is heavily restricted and uni-directional from the Modern DNA lab. In the lab, all members must wear full body Tyvek suits, hair nets, hoods, face masks, arm guards, booties, and gloves. To limit the possibility for outside contamination, all surfaces are bleached daily, and all instruments are subjected to UV light at least 15 minutes prior to use. This method is nondestructive, as it does not destroy the artifact.

Process:

1. Tools are scrubbed under lukewarm water and sprayed with a 10% bleach solution.
2. They are then transferred to polypropylene bags filled with a 5% ammonium hydroxide solution, and left to soak for 30 minutes.
3. Sonication is performed at 50 hrtz for 5 minutes.
4. Bags are drained, and samples are placed in a Vaccufuge to evaporate the Ammonium hydrooxide.
5. We add EDTA and PB buffer to the supernatant, which is then washed over filters in an Amnicon Centrifuge.
6. Samples are cleaned with QIAGEN Mini Elute PCR Purification kit.
7. Samples are then PCR amplified with primers for Cytochrome B and 16s to target specie to species specific regions of mitochondrial DNA.

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